FORM PTO-1390 (Modified) (REV 10-95) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR 1.5)
678-99	09/463801

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP98/04726	29 July 1998	30 July 1997

TITLE OF INVENTION

MEDIUM FOR PRODUCING AND/OR TREATING ALCOHOLIC BEVERAGES, ESPECIALLY WINE OR SPARKLING WINE, AS WELL AS ITS APPLICATIONS

APPLICANT(S) FOR DO/EO/US

Holger Lowel, Rainer Pommersheim

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ A copy of the International Search Report (PCT/ISA/210).
8. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U S.C. 371 (c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
10. ☒ An oath or declaration of the inventor(s) (35 U.S C. 371 (c)(4)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 13 to 18 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
    A **SECOND** or **SUBSEQUENT** preliminary amendment.
16. ☐ A substitute specification.
17. ☐ A change of power of attorney and/or address letter.
18. ☒ Certificate of Mailing by Express Mail
19. ☐ Other items or information:

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/463801	PCT/EP98/04726	678-99

20. The following fees are submitted:.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

		CALCULATIONS PTO USE ONLY
☐	Search Report has been prepared by the EPO or JPO	**$840.00**
☐	International preliminary examination fee paid to USPTO (37 CFR 1.482)	**$670.00**
☐	No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2))	**$760.00**
☒	Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2) paid to USPTO	**$970.00**
☐	International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4)	**$96.00**

ENTER APPROPRIATE BASIC FEE AMOUNT =	**$970.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).	**$0.00**	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	40 - 20 =	20	x $18.00	**$360.00**	
Independent claims	1 - 3 =	0	x $78.00	**$0.00**	
Multiple Dependent Claims **(check if applicable)**.			☒	**$260.00**	
TOTAL OF ABOVE CALCULATIONS			=	**$1,590.00**	
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) **(check if applicable)**.			☐	**$0.00**	
SUBTOTAL			=	**$1,590.00**	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)).			+	**$0.00**	
TOTAL NATIONAL FEE			=	**$1,590.00**	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).**			☐	**$0.00**	
TOTAL FEES ENCLOSED			=	**$1,590.00**	
				Amount to be: refunded	**$**
				charged	**$**

☒ A check in the amount of **$1,590.00** to cover the above fees is enclosed.

☐ Please charge my Deposit Account No. in the amount of to cover the above fees.
A duplicate copy of this sheet is enclosed.

☒ The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. **16-0750** A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

John F. McNulty, Esquire
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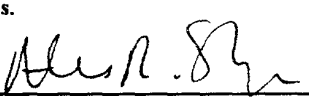

SIGNATURE

Alex R. Sluzas

NAME

28,669

REGISTRATION NUMBER

January 27, 2000

DATE

PATENT

IN THE UNITED STATES PATENT OFFICE

Serial No.: Not yet assigned

Filed: Herewith

For: MEDIUM FOR PRODUCING AND/OR TREATING ALCOHOLIC BEVERAGES, ESPECIALLY WINE OR SPARAKLING WINE, AS WELL AS ITS APPLICATIONS

Inventor: Holger Lowe
Rainer Pommersheim

Atty Doc. No.: 678-99

PRELIMINARY AMENDMENT

Assistant Commissioner
for Patents
Washington, D.C. 20231

Dear Sir:

In the specification:

Page 11, last line, after "figure" insert --1--.

In the claims:

Claim 5, line 1, delete "or 4";

Claims 7, 8, 9, 10, 11, 12, 14 and 15, line 1 of each, after "claims" insert --1-2--;

Claim 18, line 1, delete "or 17";

Claims 20 and 21, line 1 of each, change "19" to --2--.

The application should be amended in accordance with the "Amended Primary Claims" dated 7/23/99, enclosed.

Please further amend the application by adding the enclosed Abstract thereto, as indicated on the attached sheet hereto.

REMARKS

The above-identified amendments have been made to place the application in better form for examination and to eliminate multiple dependent claims from depending on multiple dependent claims.

Respectfully submitted,

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Atty. Doc. 678-99

VERIFICATION OF TRANSLATION

I, Leslie Pahl, a citizen of the United States of American, residing at the address indicated below, hereby declare:

That I am knowledgeable in the English and German languages;

That I can translate from German to English;

That the English translation attached hereto is a true and complete translation of the German language International Application PCT/EP98/04726.

That all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 12/26/99

Leslie Pahl

Address: 334 63rd St.
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94618

Atty Doc. 678-99

Medium for Producing and/or Treating Alcoholic Beverages, Especially Wine or Sparkling Wine, as Well as Its Applications

Description

The invention refers to a medium for producing and/or treating alcoholic beverages, especially wine or sparkling wine, in accordance with the generic meaning of the principal claim as well as its applications.

Various species of microorganisms, especially yeast used for alcohol fermentation, are employed in the production of alcoholic beverages, especially of wine or sparkling wine. To optimize the results, other species of microorganisms and enzymes are added to the product or its preliminary stages. Thus, for example, lactic acid bacteria serve to break down malic acid and pectins accelerate must clarification.

The process of alcohol fermentation can be interrupted by such processes as rapid cooling down, addition of sulfur dioxide, or filtration. These processes for inactivating the yeast are, however, costly, only imprecisely controllable, and can impair the product's quality. Thus, a time-delayed use of various yeast species turns out to be costly.

After handling, for example, a wine with lactic acid bacteria to reduce the acid content, the added microorganisms have to be separated, which takes place with the relatively small lactic acid bacteria via membrane filtration. However, in this connection there is not always a guarantee of complete removal of microorganisms. Lactic acid bacteria remaining in the wine can transform glucose into acetic acid, thus ruining the wine.

Enzymes such as proteases to break down peptides and proteins are added to the product or its preliminary stages in liquid form. The inactivation of enzymes as a rule takes place by heating, whereby spoilage to the product can result, as well as excluding a recycling of in part expensive enzymes.

There are mediums known in sparkling wine production for bottle fermentation that consist of yeast's immobilized in alginate beads (G. Troost, et al., Sekt, Schaumwein, Perlwein [Champaigne, Sparkling Wine, Perlant Wine] Stuttgart 1995 and DE 39 08 997 A1). With this, the time-consuming manual riddling of the fine yeast deposits could be replaced by quickly sinking the alginate beads in the champagne bottle. The disadvantage of such mediums, in which the beads are not surrounded by a cell-free membrane cartridge, however, is that they do not show any high mechanical stability, and cannot sufficiently prevent growth of especially the relatively smaller microorganisms, whereby the microorganisms can remain in the product after separation of the beads. A multiple use of such medium can only be realized with difficulty. Furthermore, enzymes are usually not immobilizable because of their small quantity in such alginate beads.

US 4,996.150 describes a process for the microencapsulation of biocatalysers, preferably yeast, as well as their use in the continuous production of ethanol. The biocatalysers are contained in a matrix of an anionic polysaccharide and a cationic polymer. Here, too, the microcapsules do not display any cell-free membrane cartridge, so that yeast growth cannot be sufficiently prevented. Additionally, it is hardly possible to immobilize the smaller biocatalyzers, such as enzymes, with sufficient surety.

US 4,659,662 describes a process for producing alcoholic beverages or bioalcohol by using yeast-containing microcapsules. The yeast can be embedded in a matrix material that is additionally surrounded by a membrane cartridge, one that, however, is the same as the matrix material. Yeasts immobilized in calcium alginate were named as an example. The membrane cartridge, however, consists of just a single layer and is, moreover, infiltrated with cells, that is, not cell-free. Thus, growth of the yeasts cannot be sufficiently prevented and a sufficient immobilization of enzymes with long-term stability is hardly possible.

DE 34 32 923 C2 concerns biocatalyzers with immobilized cells that are surrounded by a single-layer, cell-free membrane cartridge. The membrane cartridge, which

surrounds the inner capsule and is not cell permeable, can consist of a interlinking ionic or covalent gel.

Champaigne production will be used as an applications example. The membrane cartridge consists preferably of the same substance as the matrix material in the inner capsule. The interlinkingmedium, for example calcium ions, containing a surplus of the beads, here out of calcium malginate, is again put into a cell-free substance, here an alginate solution, that forms the membrane cartridge. The disadvantage with this is that liquefaction of the capsule's contents is not possible without dissolving the membrane cartridge. Especially with enzymes, however, a liquifacted inner capsule facilitates the preservation of natural conformity and thus the enzyme activities. Beyond this, such membrane cartridges hardly allow for attaining standards such as targeted permeability and a sufficiently high mechanical stability.

The purpose of the invention is to make available a medium for producing and/or treating alcoholic beverages, especially wine or sparkling wine, according to the generic term of the primary claim, in which the cells or enzymes are permanently immobilized, in which the permeability and the mechanical stability of the membrane cartridge is adjustable, and in which the contents of the microcapsules can be liquefied.

The invention has the further purpose of demonstrating applications relating to the medium according to the invention.

The purpose will be evidenced through a medium with the characteristics of claim 1 as well as through the applications according to claims 20 and 21, whereby the sub-claims address the advantageous aspects of the invention.

The species of microorganisms and/or enzymes used in the production and/or treating of alcoholic beverages, especially wine or sparkling wine, are immobilized in that they are contained in the inner capsule, and that a membrane cartridge completely surrounds the inner capsule. The membrane cartridge for these microorganisms or

enzymes is not permeable, thus preventing the escape of the microorganisms or enzymes. In order to ensure a substance conversion, the membrane cartridge for the substances to be converted (output product) is permeable, also included in this are the nutrients necessary for the microorganisms, for example, glucose; the membrane cartridge is also permeable to at least a part of the produced or converted substances (products), alcohol and carbon dioxide, for example. The requirements of permeability and mechanical stability were fulfilled by the membrane cartridge that has at least two layers, radially arranged on top of each other, whereby each layer completely encloses all of the radially arranged layers beneath it. The advantage here is that the individual layers are bound together ironically and/or covalently.

Only this multi-layered construction allows the permanent immobilization of cells or enzymes for the production and/or treating of alcoholic beverages, such as wine or sparkling wine. So, for instance, this effectively prevents an outgrowth of yeast's and with this an accompanying damage of the microcapsules. With the high mechanical stability, the microcapsules can be used in larger bioreactors without squashing or popping the microcapsules. This increased stability also enables the inner capsule to liquefy, without the microcapsules becoming too mechanically instable in the process. Additionally, the permeability of the membrane cartridge can be selectively adjusted by a suitable selection of at least two layers, which is of decisive importance for immobilizing the enzymes.

Preferably, not only the outer layers, but also the innermost layer of the membrane capsule evidence none of the cells or enzymes contained in the inner part of the microcapsules.

Preferably, at least two layers of the membrane cartridge consist of different substances. Thus, for example, an outer layer (support layer) can consist of a substance that ensures a high level of mechanical stability, while an inner layer (control layer) consists of a substance that enables a selective adjustment of this layer's permeability and with that the membrane cartridge's permeability.

After an advantageously executed form, the cells or enzymes contained in the inner microcapsules are embedded in a matrix. This matrix can be constructed from an alginate combination with a polyvalent cation, for example, calcium, strontium, barium, aluminum and/or iron.

According to another advantageously executed form, the cells or enzymes in the inner microcapsules freely move about in a fluid, which is especially advantageous for maintaining the enzyme's natural conformation. With this, the cells' or enzymes' natural activity is maintained despite being immobilized. If, by means of an interlinking medium, the microcapsules are produced by the precipitation of beads of a solution containing cells or enzymes, the inner capsule can again be liquefied after the membrane cartridge's layers are fixed. If, for example, the matrix substance in the inner capsule consists of calcium alginate, then the polyvalent metallic cation can be exchanged for a monovalent cation, for example, natrium or calcium, in order to again liquefy the inner microcapsule. It is thus preferable that at least one layer of membrane capsule consists of one of the different substances that embed the cells or enzymes and that constitute the matrix. As far as known microcapsules are concerned, where the single layer membrane cartridge consists of the same substance as the matrix in the inner capsule, this liquefaction of the inner capsule is not possible, since the membrane cartridge would in this case also dissolve.

In the case of an interlinkable substance using polyvalent cations, a further advantage of exchanging the polyvalent cations for monovalent cations is that polyvalent cations, such as calcium, are as a rule undesirable in wine production. Thus, for example, a matrix of calcium alginate can become liquefied by introducing the microcapsules into a watery solution containing natrium citrate, which creates an exchange of calcium ions for natrium ions. These microcapsules, such as those used in wine production for example, preferably will bond polyvalent ions from the substrate to be converted, such as grape juice, which has an advantageous effect. If these microcapsules' matrix again shows a too-high content of polyvalent cations,

then the matrix can be regenerated again through treating it with a solution containing monovalent cations.

Microcapsules with a membrane cartridge out of one layer as well as out of multiple layers are known in the medical field to immobilize cells or enzymes. Thus, Langerhansian cells are encased in a microcapsule, with the membrane cartridge being constructed from a layer consisting of alginate and poly-L-lysine. (F. Lim et al., Science, 210 (1980) 908-910). EP-A-681834 described microcapsules, with the membrane cartridge being constructed of numerous layers, for introduction into living organism tissue.

The requirements of such microcapsules are tissue tolerance, a low level of immune reaction, and ability to introduce them into living organism tissue.

By optimizing microcapsule construction with a multi-layered membrane cartridge with a view to the production or treating of alcoholic beverages, suitable microorganisms, such as yeast used in alcohol fermentation or lactic acid bacteria, and/or enzymes, can be immobilized advantageously. Particularly in the case of immobilizing yeast for alcohol fermentation, stability has to be ensured, despite the production of carbon dioxide and a rapid growth of yeast cells. This is achieved through microcapsules with membrane cartridges that have at least two layers arranged radially above each other.

The medium according to the invention has an advantage over the already familiar mediums [used] in the production of alcoholic beverages in that the encapsulated cells or enzymes are permanently immobilized and, because of the simple operations process, can be easily measured out and added and can also be simply, quickly, and completely removed from the product. This allows for a specific influence of the individual production steps without impairing product quality. Additionally, microcapsules containing cells or enzymes can be reused, which leads to cost savings where expensive enzymes are used. Further, a good substance exchange, especially the gas exchange in alcohol fermentation, is ensured because of the hollow spaces

present between the capsules even where sedimentary microcapsules are involved, as well as because of the microcapsules' easy mobility in a fluid substance.

It can be advantageous to make the membrane capsule impermeable to those active substances and/or microorganisms situated outside the microcapsule that could impair the activity of the cells or enzymes contained in the inner capsule. Thus, some species of yeast used in wine production produce toxins that are harmful to other species of microorganisms. By using microcapsules having a membrane cartridge that is not permeable by such toxins, such microorganisms or enzymes can be introduced together, for example, into wine production.

After a preferred implementation method, the microcapsules contain as microorganisms at least one of the yeast species employed in the wine production process.

After a further preferred implementation method, there is in the microcapsules at least one species of the lactic acid bacteria that are used in biological acid breakdown in wine production.

Processes to deacidify wine by using cells of the Leiconostoc oenos species immobilized in calcium alginate are known from US 4,380,552. These beads, however, have no membrane cartridge, thus not ensuring sufficient stability and an outgrowth of cells.

Advantageously suited as a method of producing or treating alcoholic beverages are microcapsules that contained enzymes such as pectases, glycosides, β-glucosidases, proteases, and/or glucose-fructose-isomerases used especially in wine production.

It can be advantageous to immobilize cells as well as one or more enzymes in a microcapsule. Firstly, this can make implementation easier, and secondly, this can especially increase productivity if a product of the microorganism or enzyme is

further converted by the other microorganisms or enzymes contained in the microcapsule. Thus, species of yeast and their treatment substances such as the yeast cell wall preparations and/or glucose-fructose-isomerases used in wine production can be advantageously immobilized together in a microcapsule. Aside from an increase in activity of the yeast used, the quantities of, in part, expensive treatment substances to be used can be reduced.

Activity of the immobilized cells or enzyme-increasing substances can also be encapsulated in a microcapsule. It is thus, for example, known that the activity of lactic acid bacteria decreases with an increase in acid content. By including a cation exchanger in a microcapsule containing lactic acid bacteria, the pH-level can be increased at the site of lactic acid bacteria through an exchange of hydrogen ions for, for example, potassium ions, thus increasing the activity of lactic acid bacteria. Further examples of activity-increasing substances are vitamins, such a vitamin B_1, or growth-promoting proteins.

Preferably, at least one layer of the membrane cartridge will consist of at least one polymer. Suitable as a polymer would preferably be a polyelectrolyte complex that consists of at least one polycation and one polyanion. Suitable polyanions are, for example, polyacrylic acid, polymethacrylic acid, polyvinylsulfonic acid, polyvinylphosponic acid, alginate acid, cellulose derivatives, especially carboxylmethyl cellulose or cellulose-sulfuric acid ester, shellac or shellac components such as aleuritin acid or shellol acid. Suitable polycations are, for example, polyethylenimine, polydimethyl dialylammonium, poly-L-lysin or chitosan.

The polyanions or polycations will preferably have a mid-polymerization level of above 100, preferably from 100 to 15,000. For the specific adjustment of the membrane cartridge's permeability, the polymers preferably used for the layer that determines the permeability (regulating layer) are polymers with a narrow distribution of molecular mass, for example, synthetic polyelctrolytic complexes from polyacrylic acid or polymethacrylic acid with polyethylnimine. Thus, the permeability of a small

protein having a quantity of about 60 kD is attained through a regulating layer of polyethylenimine (PEI) (molecular mass 1,000,000) and polyacrylic acid (PAS).

Table 1 shows the portion of protein diffused from the microcapsule in connection with the level of PAS polymerization after 60 hours of riddling.

Regulating Layer	Molecular Mass of NaPAS	Level of Polymerization	Permeability After 60 Hours
PEI/PAS	20,000	215	8.4%
PEI/PAS	60,000	645	18.9%
PEI/PAS	170,000	1828	93.1%

Table 1. Permeability of the regulating layer for a 60 kD protein in connection with the molecular mass of a polyanion as natrium-polycrylate (NaPAS).

As seen in Table 1, the permeability of the regulating layer is determined by the polyions, in this case a polyanion polyacrylic acid that has a small level of polymerization, while the counter ions, in this case the polycation polyethylenimine with a high level of polymerization, form the framework. Where there is the same counter ion, the polyelectrolytic complex with a poly ion with a high level of polymerization has a greater strength than those with a poly ion with a low level of polymerization. As framework-forming counter ions that take up the polyions with a lower level of polymerization, polyions with a polymerization level of 50,000 are preferred.

To immobilize the enzymes, at least one regulating layer with a polymerization level of the polyanions or polycations of 100 to 1,000 is preferred, whereby the framework-forming counter ions, polycations or polyanions have higher polymerization levels. To immobilize microorganisms such as yeast, in contrast, higher polymerization

levels are sufficient; here polymerization levels of 1,000 to 15,000 of polyanions or polycations in the regulating layers are preferred, whereby here, too, the framework-forming counter ions, polycations or polyanions, have a higher level of polymerization. Polyions with high polymerization levels have the advantage of greater strength in the relevant layer.

For the strength-determining layer (support layer), polyelectrolyte complexes of synthetic polycations or polyanions with high polymerization levels of over 10,000 are preferably used. Natural polycations and anions such as alginate acid and chitosan or cellulose derivatives can also be used advantageously; with these, a wide molecular mass distribution does not have a disruptive effect.

Table 2 shows examples for substances in the membrane cartridge layers, whereby each first row lists whether this structure is preferably suited for immobilization of yeast and/or enzymes. The core can have an alginate for immobilization that can be liquefied after the layers have been put in. In each Table 2 column, the layer structure from inner to outer is given, With a larger number of layers, for example 4 or more layers, a still greater membrane cartridge stability can be achieved.

	Yeasts	Yeasts	Yeasts and/or Enzymes	Yeasts and/or Enzymes	Enzymes	Enzymes
1. Layer	PEI/CMC	PEI/CMC	Chit/PAS	Chit/PAS	PEI/PAS	PEI/PAS
2. Layer	PEI/CMC	PEI/CMC	Chit/CMC	Chit/CMC	PEI/CMC	PEI/CMC
3. Layer		PEI/CMC		Chit/CMC		PEI/Alg

Table 2. Examples of the Membrane Cartridge's Layer Structure with Immobilized Yeasts and/or Enzymes (Alg = Alginate, Chit = Chitosan, CMC = Carboxymethyl cellulose, PAS = Polyacrylic acid, PEI = Polyethylimine).

In addition, natural rubber, polystyrene and/or polymethylmethacrylate or a mixture thereof with one or more polyelectrolyte complexes are suitable as polymers for building a membrane cartridge layer.

The invention also concerns two applications for the medium according to the invention. Thus the medium can be used in beer production. Here, microcapsules are to be used with cells from one or more yeast species used in beer production. Likewise, the medium can be used for producing low-molecular alcohol such as methanol and/or ethanol, whereby microcapsules with yeasts suitable for high-yield alcohol production are used.

To produce and/or treat wine, for example, using the medium according to the invention, the microcapsules are to be put into a grape, berry and/or other fruit juice, apple juice, for example, or into a wine. The microcapsules stay in the solution until partial or complete conversion, such as alcohol fermentation, has taken place. Finally, the microcapsules are removed from the solution.

As opposed to already-known mediums in which the microorganisms or enzymes used have to be filtered off, this medium has the decisive advantage of allowing the cells or enzymes used in the production or treatment process to be simply, quickly, and completely removed in the last step of the process. With this, the time that microorganisms or enzymes remain in the solution can be precisely adjusted.

Because of the microcapsules' size, diameters of one-half or less millimeters are preferred the microcapsules can be simply and completely removed from the solution. Mechanical processes are appropriate here, for example by using a sieve, or decanting the liquid remaining after prior sedimentation of the microcapsules. In these removal processes, the microcapsules are not destroyed, so that they can be reused if need be after being stored in the interim in a nutritive solution. Especially in the case of expensive enzymes, production costs can be reduced.

Advantageously, at least in the area of microcapsules, the solution is set to just the temperature that makes the cells or enzymes optimally active, whereby a possible influence in the product quality is to be considered.

Various microorganisms or enzymes can be used in microcapsules simultaneously and/or time-delayed. In the case of time-delayed usage, the various microcapsules can be successively introduced into the solution and removed together, or the processing steps of introduction, time spent in the solution, and removal are successively run through multiple times with the same solution by using various cells and enzymes immobilized in the microcapsules. The use of different microorganisms or enzymes can thus be used selectively to increase the complexity of, for example, a wine.

A fluidized bed reactor, which contains the medium according to the invention, can be used as a bioreactor for production of low-molecular alcohol, especially ethanol, or alcoholic beverages, especially wine or sparkling wine. Suitable reactor types are also listed in Lüders, "Technologie mit immobilisierten Hefen" [Technology with Immobilized Yeasts], Brauwelt 1994, 57.

The bioreacter can also have at least one tube that contains the microcapsules. The two tube openings are advantageously sealed with sieves that retain the microcapsules.

This precipitates a separation of the microcapsules from the converted solution. The preferable diameter of suitable tubes is in the area of one or more centimeters.

The liquid to be converted is guided through the tubes, whereby numerous tubes can be connected parallel and/or serially to another. In the case of a simultaneous use of various microorganisms or enzymes immobilized in microcapsules, it is preferable that tubes with the same content be joined parallel to each other, whereby groups of tubes with different contents joined parallel to each other are joined together serially.

It can be advantageous to join together individual tubes serially so that the solution flows through the tubes in succession.

To optimize the activity of the microorganisms or enzymes used, it is advantageous to set the temperature of the inner part of the tubes, that the microcapsules and their surrounding solution. Here, it is advantageous for a single tube or a group of tubes to have a shared temperature-settable sleeve. The activity of the cells or enzymes can be specifically lowered by cooling the microcapsules enclosed in the tubes.

In an installation for the production of low-molecular alcohol, especially ethanol, that have at least one bioreactor, the glucose-containing liquid to be fermented goes from the storage tank into the mixer. There it is mixed with the backflow from the still and then put into the microcapsule-filled bioreactor, where the actual conversion of carbohydrates into alcohol takes place. The alcohol is then separated in the heating container and in the distillation column and gathered in the collection tank for the primary product. The separation of alcohol from the remaining liquid occurs by using the different boiling points. The remaining liquid from the still can, after cooling down in the heat exchanger, again be enriched in the mixer with fresh liquid and go renewed into the bioreactor. In order to avoid an excessive thinning of the glucose in the circulating liquid, a part of the watery portion is regularly removed and gathered as a by-product in a collection tank.

An installation for the production of alcoholic beverages has a greatly simplified structure. The liquid to be fermented is taken from a storage tank and put into an bioreactor according to the invention, where the liquid sits for a period of time or, for example, circulates through a tube reactor. The alcohol-containing product is routed from the bioreactor into a collection tank after the time required for a partial or complete conversion.

Further details of the installations can be found in the following descriptive section in which, with the drawing, an example of implementation is described in more detail. The attached figure shows the schematic structure of a continuous installation for

alcohol production. The liquid to be fermented is taken from a storage tank (1) and put into a mixer (2), where it mixes together with a liquid backflowing from the still (4). This mixture is converted into alcohol in the bioreactor (3) from the yeast immobilized in the medium according to the invention. The alcohol-containing fluid is put into a still (4). From there, an alcohol-containing gas phase is routed into a distillation column (5). There, the different boiling points of alcohol in contrast to water are used to enrich the alcohol that is gathered in a collection tank for the primary product (6). The alcohol-lacking portion of the pre-fermented liquid is taken out of the still and cooled in a heat exchanger (8) and routed back into the mixer (2). In order for the liquid in circulation to contain a minimum level of the substances to be converted, especially glucose, a part of the watery portion is regularly removed from the still (4) and gathered as a by-product in a collection tank (7).

Example of Microcapsule Production:

5 g natrium alginate (Kelco Co., Hamburg) are dissolved in 700 ml water. Then 70 g of dry yeast was subsequently stirred into this solution (Oenoform, Erbslöh Co., Geisenheim). This suspension was dropped into a 0.6% solution of calcium chloride. After a few minutes of curing time, the beads containing the yeast cells in a calcium-alginate matrix were first washed with water and subsequently with a watery 0.05% solution of polyethylenimine (mid-range molecular mass 1 mil, Fluka Co.) and was afterwards washed with a watery 0.06% of carboxymethyl cellulose (mid-range viscosity, Fluka Co.). Subsequently, the microcapsules thus obtained were washed with water and once again put into the polyethylynimine and carboxymethyl cellulose solution. After being cleaned with water, the microcapsules were stored in water. The microcapsules have a two-layered membrane cartridge, whereby each layer consists of the polyelectrolyte complex of polyethylynimine and carboxymethyl cellulose. Because the calcium alginate beads showing cells were produced first and because the layers were subsequently applied to the membrane cartridge, the membrane cartridge layers showed no yeast cells that could grow out of the microcapsules. The yeast thus immobilized showed the same activity as in yeast immobilized in unlayered calcium-alginate beads.

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EM 49-97
7/23/99

Amended Primary Claim

1. Medium for producing and/or treating alcohol-containing beverages, especially wine or sparkling wine,

consisting of microcapsules that each show at least one membrane cartridge that completely encloses the inner capsule, whereby the inner capsule cells include at least one species of microorganisms and/or one or more enzymes, and

whereby the membrane cartridge is not permeable to the cells or enzymes enclosed in the inner capsule, and

whereby the membrane cartridge is permeable to the output product to be converted by the cells or enzymes and to at least one part of the products converted by the cells or enzymes.

Characterized in that

the membrane cartridge has two layers radially arranged above one another, whereby each layer completely encloses all of the layers radially arranged below it, and whereby the layers do not exhibit any cells or enzymes enclosed in the inner part of the capsule.

Patent Claims

1. Medium for producing and/or treating alcohol-containing beverages, especially wine or sparkling wine,

consisting of microcapsules, that each show at least one membrane cartridge that completely encloses the inner capsule, whereby the inner capsule cells include at least one species of microorganisms and/or one or more enzymes, and

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whereby the membrane cartridge is permeable to the output product to be converted by the cells or enzymes and to at least one part of the products converted by the cells or enzymes.

Characterized in that

the membrane cartridge has two layers radially arranged above one another, whereby each layer completely encloses all of the layers radially arranged below it.

2. Medium according to claim 1, characterized in that at least two layers of the membrane cartridge consist of different substances.

3. Medium according to claim 1 or 2, characterized in that the cells or enzymes contained in the inner microcapsule are embedded in a matrix.

4. Medium according to claim 3, characterized in that the matrix shows an alginate bond of a polyvalent cation.

5. Medium according to Claim 3 or 4, characterized in that at least one layer of the membrane cartridge consists of one of the different substances that embed the cells or enzymes and that constitute the matrix.

6. Medium according to Claim 5, characterized in that the matrix in the inner part of the microcapsule is liquefied.

7. Medium according to one of the previous claims, characterized in that the layers are bonded covalently and/or ionically with each other.

8. Medium according to one of the previous claims, characterized in that the membrane cartridge is not permeable to the active substances and/or microorganisms found outside the microcapsule, which impair the activity of the cells or enzymes contained in the inner capsule.

9. Medium according to one of the previous claims, characterized in that the inner part of the microcapsule contains cells of at least one species of yeast used in alcohol fermentation, preferably in wine production.

10. Medium according to one of the previous claims, characterized in that the inner part of the microcapsule contains cells of at least one species of lactic acid bacteria used in the biological acid breakdown process in wine treatment.

11. Medium according to one of the previous claims, characterized in that the inner part of the microcapsule contains one or more enzymes from the group of pectases, glycosides, β-glucosidases, proteases, and/or glucose-fructose-isomerases.

12. Medium according to one of the previous claims, characterized in that the inner part of the microcapsule contains cells from at least one species of microorganisms and at least one enzyme.

13. Medium according to claim 12, characterized in that the inner part of the microcapsule contains at least one species of yeast used in wine production as well as at least one yeast cell wall preparation and/or a glucose-fructose-isomerase.

14. Medium according to one of the previous claims, characterized in that the inner part of the microcapsule contains, apart from the cells or enzymes, at least one substance that increases the activity of the cells or enzymes.

15. Medium according to one of the previous claims, characterized in at least one layer of the membrane cartridge is constructed of at least one polymer.

16. Medium according to claim 15, characterized in that that polymer is a polyelectrolyte complex.

17. Medium according to claim 16, characterized in that the polyelectrolyte complex has a polyanion from the group of polyacrylic acid, polymethacrylic acid, polyvinylsulfonic acid, polyvinylphosponic acid, alginate acid, cellulose derivatives, especially carboxylmethyl cellulose or cellulose-sulfuric acid ester, shellac or shellac components such as aleuritin acid or shellol acid and at least one polycation from the group of polyethylenimine, polydimethyl dialylammonium, chitosan, or poly-L-lysin.

18. Medium according to claim 16 or 17, characterized in that the polyanion or the polycation has a mid-range polymerization level of from 100 to 15,000, whereby the polycation or polyanion as counter-ion has a mid-range polymerization level of over 50,000.

19. Medium according to claim 15, characterized in that the polymer polystyrol, polymethyl acrylate and/or natural rubber or a mixture thereof is with one or more polyelectrolytic complexes.

20. Application of a medium according to claims 1 through 19 for the production of beer, characterized in that the microcapsules contain cells of one or more of the yeast species used in beer production.

21. Application of a medium according to claims 1 through 19 for the production of low-molecular alcohol, preferably from ethanol, characterized in that the microcapsules contain cells of one or more of the yeast species that enable high yields of alcohol production.

MEDIUM FOR PRODUCING AND/OR TREATING ALCOHOLIC BEVERAGES, ESPECIALLY WINE OR SPARAKLING WINE, AS WELL AS ITS APPLICATIONS

Abstract of the Disclosure

The invention relates to an agent used in the production and/or processing of alcoholic beverages, in particular wine or sparkling wine. This agent is composed of microcapsules which each have an enveloping membrane that encloses the capsule interior completely. The capsule interior contains cells of at least one species of micro-organism, such as yeast or lactic acid bacteria, and/or one or more enzymes. The invention seeks to produce microcapsules having the following characteristics: ability to permanently immobilize the cells or enzymes; presence of enveloping membranes with adjustable transparency and mechanical stability, in accordance with need; liquefiable content of the microcapsules. To this end, the envelope membrane is composed of at least two radially superimposed layers. Each layer encloses all the layers arranged beneath it completely. The invention also relates to the use of this agent in the production of beer and low-molecular alcohols.

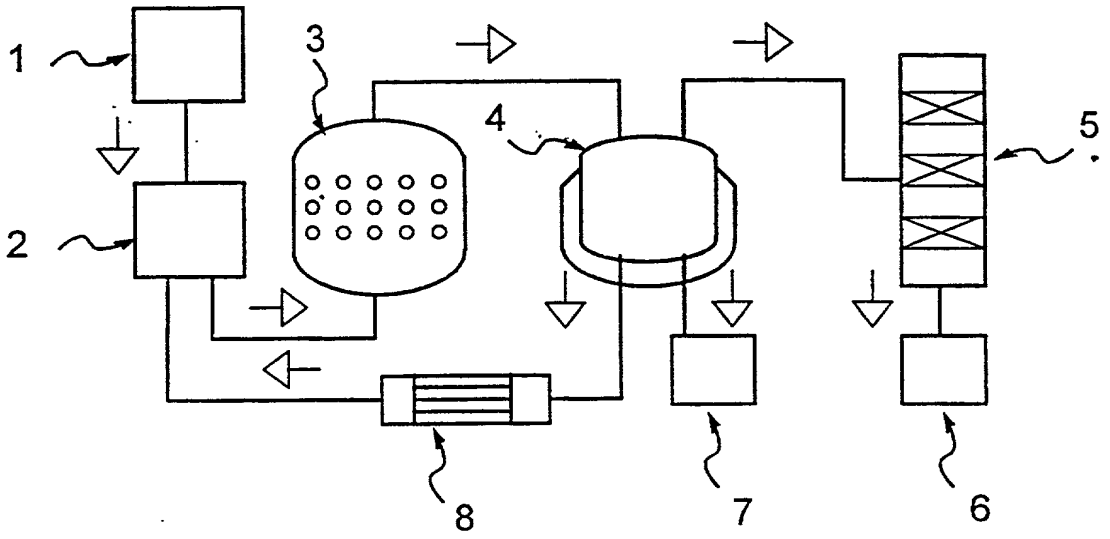

Fig. 1

Attorney Doc.: 678-99

DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled AGENT FOR PRODUCING AND/OR PROCESSING ALCOHOLIC BEVERAGES, IN PARTICULAR WINE OR SPARKLING WINE, AND USE OF SUCH AGENT the specification of which

is based on PCT/EP98/04726 filed July 29, 1998 which is based on German Patent Application 197 32 710.9 filed July 30, 1997.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)			Priority Claimed	
PCT/EP98/04726	International	29 July 1998	X	—
(Number)	(Country)	(Day/Month/Year Filed)	Yes	No
19732710.9	Germany	30 July 1997	X	—
(Number)	(Country)	(Day/Month/Year Filed)	Yes	No

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which

occurred between the filing date of the prior application and the national or PCT international filing date of the application:

(Application Serial No.)	(Filing Date)	(Status) (patent, pending, abandoned)

I hereby appoint the following attorneys and/or agents to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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